



Research article

Sampling methods and approaches to inform standardized detection of marine alien fouling species on recreational vessels

Koebräa Peters^a, Kerry J. Sink^b, Tamara B. Robinson^{a,*}^a Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Matieland, 7602, South Africa^b Marine Programme, South African National Biodiversity Institute, Kirstenbosch Research Centre, Private Bag X7, Claremont 7735, South Africa

ARTICLE INFO

Keywords:

Alien species
Biofouling
Secondary spread
Monitoring
Yachts

ABSTRACT

Recreational vessels are important contributors to the spread of marine alien species, particularly in relation to secondary spread within novel regions. As such, these vessels should be considered a monitoring priority. The aim of this study was to identify a preferred method for monitoring recreational vessels for alien species, while simultaneously developing a framework that enables managers to objectively choose the most effective sampling approach given their financial constraints. Divers and a remotely operated vehicle (ROV) were considered in relation to four sampling approaches i.e. meanders, transects, inspection of niche areas and the collection of quadrats. Each was applied to the same 53 vessels which represented a spectrum of hull fouling cover. The most effective methods were diver scrape quadrats (Range of alien species numbers per quadrat: 0–9, Total alien species: 20) and inspections of niche areas (Range of alien species numbers: 0–5, Total alien species: 9). All methods employed using an ROV had low efficacy and incurred high costs. While scrape samples were one of the most expensive methods, this was offset by the lowest cost per species detected. Thus, it is recommended that monitoring programmes utilize scrape samples and niche area inspections, but when faced with financial constraints, diver meanders and niche inspections offer sound alternatives for detecting alien species.

1. Introduction

Alien species are recognized as an important threat to biodiversity and ecosystem functioning in terrestrial, freshwater and marine environments (Mack et al., 2000; Marchi et al., 2011; Simberloff et al., 2013; Chan and Briski, 2017). In the marine environment, such species are transferred by a variety of vectors including ballast water (Adebayo et al., 2013), biofouling (Williams et al., 2013), aquaculture (Grosholz et al., 2015), the aquarium trade (Holmberg et al., 2015) and canals (Galil et al., 2015). Of these vectors, biofouling (i.e. the attachment or growth of biota on the submerged sections of hulls and niche areas of vessels (Coutts and Taylor, 2004)) has become dominant in recent years (Hewitt et al., 2009; Williams et al., 2013) with the role of fouling on recreational vessels becoming increasingly recognized (Davidson et al., 2010; Hewitt et al., 2007; Peters et al., 2017). Species are able to establish and accumulate on these vessels during their long stationary periods in marinas (Hewitt et al., 2009), a process that is aided by inefficient or ill-maintained anti-fouling paint (Floerl and Inglis, 2005). While it is not an easy task to assign an unequivocal link between an already established alien species and the vector through which it arrived (Minchin, 2007a), recreational vessels have played a key role in

the spread of some marine algae and invertebrate species (Hewitt et al., 2007; Minchin et al., 2006). One of the best documented cases of yacht transfer is that of the mussel *Mytilopsis sallei* that was introduced to Darwin Harbour estuary (Willan et al., 2000). This introduction culminated in one of the first successful large-scale marine eradication attempts, following its early detection and the ensuing rapid response by authorities (Bax et al., 2002).

The example of *M. sallei* highlights the need to effectively survey small vessel fouling assemblages to detect alien species. Sampling of subtidal communities has been undertaken using a variety of techniques, ranging from visual to extractive approaches (Mallet and Pelletier, 2014). For fouling assemblages, approaches that have been used include visual observations from the surface (Brine et al., 2013; Floerl et al., 2005), subtidal visual surveys by divers that may include the taking of photographs and videos (Chapman et al., 2013; Coutts and Taylor, 2004; Davidson et al., 2009), the use of pole cameras operated from the surface (Brine et al., 2013; Davidson et al., 2010; Zabin et al., 2014), the use of remotely operated devices (Davidson et al., 2009; Lee and Chown, 2009; Needles and Wendt, 2013) and extractive sampling whereby samples are collected and then processed by taxonomic experts (Chapman et al., 2013; Coutts and Dodgson, 2007; Davidson et al.,

* Corresponding author.

E-mail address: trobins@sun.ac.za (T.B. Robinson).<https://doi.org/10.1016/j.jenvman.2018.09.063>

Received 22 February 2018; Received in revised form 5 September 2018; Accepted 20 September 2018

Available online 01 October 2018

0301-4797/© 2018 Elsevier Ltd. All rights reserved.

2010; Zabin et al., 2014). These methods require differing levels of expertise, with varying associated costs. Nonetheless, the success of detecting alien species may vary depending on the sampling approach applied, and this is unavoidably linked to the availability of resources (see Mallet and Pelletier, 2014). Non-invasive visual techniques, such as video surveys, enable the collection of large datasets that can be time-efficient (Lam et al., 2006). In contrast, the collection of samples allows for accurate identification and detection of smaller and inconspicuous organisms, ultimately resulting in higher species detection rates (Peters et al., 2014). Although these subtidal sampling techniques have been applied in various contexts, few studies have quantitatively compared the efficacy of these methods for assessing fouling assemblages (but see Beaumont et al., 2007), and none have considered their applicability for detecting alien species on recreational vessels. Notably, the relative costs involved with using these kinds of techniques have not been objectively compared (Mallet and Pelletier, 2014), despite the important implications that this has for management organizations tasked with monitoring for marine alien species.

Besides moving between countries, recreational vessels also connect main ports to more remote regions within country borders, constituting regional secondary vectors for species introduced through primary vectors, such as ballast water and ship fouling (Clarke Murray, 2012; Wasson et al., 2001). Despite this, and the recognition of the potential importance of this vector (Clarke Murray et al., 2011; Davidson et al., 2010), there is no systematic monitoring of recreational vessels for marine alien species anywhere in the world. A precursor for the development of such a system is the establishment of an effective and cost-efficient sampling approach. While the value of such a system is clear, the development of a standardized method that could be applied across regions would be extremely beneficial. The overarching aim of this study was thus, to identify a preferred method for monitoring recreational vessels for alien species, while simultaneously developing a framework that will enable managers to objectively choose the most effective sampling approach attainable within their financial and logistical constraints.

2. Materials and methods

2.1. Study region

This study considered yachts from four marinas in the Western Cape, South Africa, to experimentally compare methods for detecting alien species on the hulls of sea-faring recreational vessels. These marinas were; Port Owen Yacht Club (32°46'56.43"S; 18°08'53.60"E), Saldanha Bay Yacht Club (33°00'37.68"S; 17°56'56.75"E), Royal Cape Yacht Club (33°55'14.15"S; 18°26'34.84"E) and False Bay Yacht Club (34°11'32.99"S; 18°26'02.20"E). All marinas are situated within or adjacent to large ports and all marinas receive both local and international yacht traffic. This study considered sailing yachts because in the South African context motorized vessels rarely move among marinas due to rough sea conditions that typify this exposed coastline.

2.2. Fouling ranks

Data were collected between December 2015 and October 2016. To gain a measure of background fouling levels, all yachts in each marina (N = 638), were visually inspected and assigned a Fouling Rank (FR). Fouling Rank is an estimated measure of the amount of biofouling present on the submerged surface of a vessel's hull, as visible from the surface. This ranking approach was adapted from the ordinal ranking system developed by Floerl et al. (2005) with the number of levels reduced to four for practical reasons (Table 1). A two-way Chi-Square test was used to determine if the number of boats differed across Fouling Ranks and marinas. All analyses, unless otherwise indicated, were undertaken in STATISTICA 13. A total of 53 yachts were sampled for alien species. This sample size was determined by the number of yacht

owners who were willing to provide access to their vessels during the study period. While a balanced sampling design with equal numbers of yachts in each Fouling Rank would have been desirable, this was impossible at the level of individual marinas.

2.3. Sampling approaches

A total of eight sampling methods were applied to each yacht, using four sampling approaches that were undertaken by both a scientific diver and a VideoRay Pro 3 Remotely Operated Vehicle (ROV). The four approaches were the Meander, Transect, Niche and Quadrat. The Meander consisted of a diver searching the submerged hull area for alien species, for a period of 6 min. The Transect involved a diver searching the circumference of the vessel (i.e. all the around the edge of the entire hull) at a distance of 50 cm below the waterline. The Niche method included inspections of all submerged niche areas of each vessel (i.e. rudder, keel, water intakes, propeller and propeller shaft). The Quadrat method involved the collection of six randomly placed photo quadrats by the ROV and those same six quadrats were scraped and all fouling collected by divers as scrape quadrats. These scrapes were later identified in the laboratory. Divers also made use of a target list of 10 alien species when searching the hull and niche areas and this list was applied in the ROV methods as well. The use of a target list was implemented in order to ensure fast and cost-effective sampling (Minchin et al., 2016) as resources for marine biosecurity are generally limited. Excepting for the processing of scrape samples that needed to be done back in the laboratory, the various methods were randomly applied to ensure no effect of sampling order on the number of species detected. The fact that various people controlled the ROV, sorted the scrape samples and undertook the diver based methods, further avoided any sampling introduced bias. Additional details regarding these methods are provided in supplementary electronic Table S1 1. For each yacht, the total number of alien species detected by each method was recorded. Additionally, for the scrape quadrats, biomass (to the nearest 0.01 g) was recorded for each species, while for the ROV photo quadrats, percentage cover was estimated.





For each method Spearman's Rank Correlations were used to detect relationships between Fouling Ranks and the number of species recorded per yacht. For Scrape and Photo Quadrats, Spearman's Rank Correlations were also used to consider correlations between the Fouling Ranks and the mean percentage cover and biomass of alien species (per yacht) respectively. The number of species recorded per yacht was analyzed using a general mixed effects model (nlme package in R) with Fouling Rank (four levels: FR 0, FR 1, FR 2, FR 3) and method (six levels: diver meander, ROV meander, diver transect, ROV transect, scrape quadrat, photo quadrat) as fixed factors and yacht as a random factor. The unequal number of yachts in the various Fouling Ranks per marina precluded the inclusion of marina as random factor. The best fit model was chosen based on Akaike Information Criteria. A Wald test was used to assess the significance of the fixed factors in the final model (Bolker et al., 2009).

In order to assess if the various methods detected different suites of species, a two factor PERMANOVA was undertaken in Primer 6 (version 6.1.16), with method considered a fixed factor and marina a random factor. PERMANOVA offers a non-parametric approach to analysis of variance for multivariate datasets (Anderson, 2001). Because the detection of species was of interest, rather than their abundance, and the fact that some methods only yielded presence/absence data, the analysis was conducted on presence/absence data only, comparing each treatment in the various marinas. To ensure a balanced design for this analysis, four yachts were randomly selected as replicates (per treatment) for each marina.

2.4. Cost of sampling approaches

In addition to the collection of biological data, the cost of each

Table 1
Fouling Ranks used in this study (adapted from Floerl et al. (2005)).

Fouling Rank	Description	Visual estimate	Example
0	No visible fouling	Nil	
1	Slime fouling, light fouling including small patches of macrofouling (1–2 taxa)	1–5%	
2	Considerable fouling, macrofouling only supported in a primary layer	6–40%	
3	Diverse assemblages covering most to all of visible hull surfaces, including primary, secondary and further layers of fouling	41–100%	

method was considered. These costs were calculated in terms of time (minutes) and money (USD). Cost in terms of time was calculated per yacht, as the time it took to set up, collect samples, move to the next boat, and to process samples. Monetary cost was also calculated per yacht as detailed in supplementary electronic Table S2. The total cost of purchasing the VideoRay Pro 4 (\$ 35 630) was used when estimating the cost of an ROV. To gain a measure of cost of an on-going monitoring programme (as opposed to a once-off study), the total cost was calculated over 3 years (the expected lifespan of a new ROV) and assumed sampling of 200 boats per year. Costs per yacht represent the average value across the four yacht clubs. For the calculation of total cost, no differentiation was made between Fouling Ranks, as a mix of Fouling Ranks occur in all marinas and logistical constraints are expected to prevent managers from deploying different methods for different Fouling Ranks within a single marina. One-Way ANOVAs were undertaken to test for differences in costs and numbers of alien species across the methods, followed by Tukey post-hoc analyses. Additionally, the cost: benefit ratio was calculated as the monetary cost per species detected by each method.

3. Results

3.1. Fouling Ranks

A total of 638 yachts were observed from the surface and assigned a Fouling Rank (FR). While the number of yachts varied across Fouling Ranks and differed among marinas (Pearson Chi-Square, $\chi^2 = 40.029$, $df = 9$, $p < 0.0001$), the patterns across Fouling Ranks remained consistent (Fig. 1). In all marinas most yachts fell within FR 1, which constitutes a slime layer and/or a fouling cover of 1–5%. This was reflected in 59% of all yachts falling within this class. Notably, only 10% and 11% of yachts were assigned to FR 0 and FR 3 respectively, while 79% had a fouling cover of 1–40% i.e. FR 1 and FR 2 combined.

3.2. Sampling approaches

A significant positive relationship was found between Fouling Rank and the number of alien species detected by all methods ($p < 0.001$ in all cases), excepting the Niche method ($p > 0.01$ for both Diver and

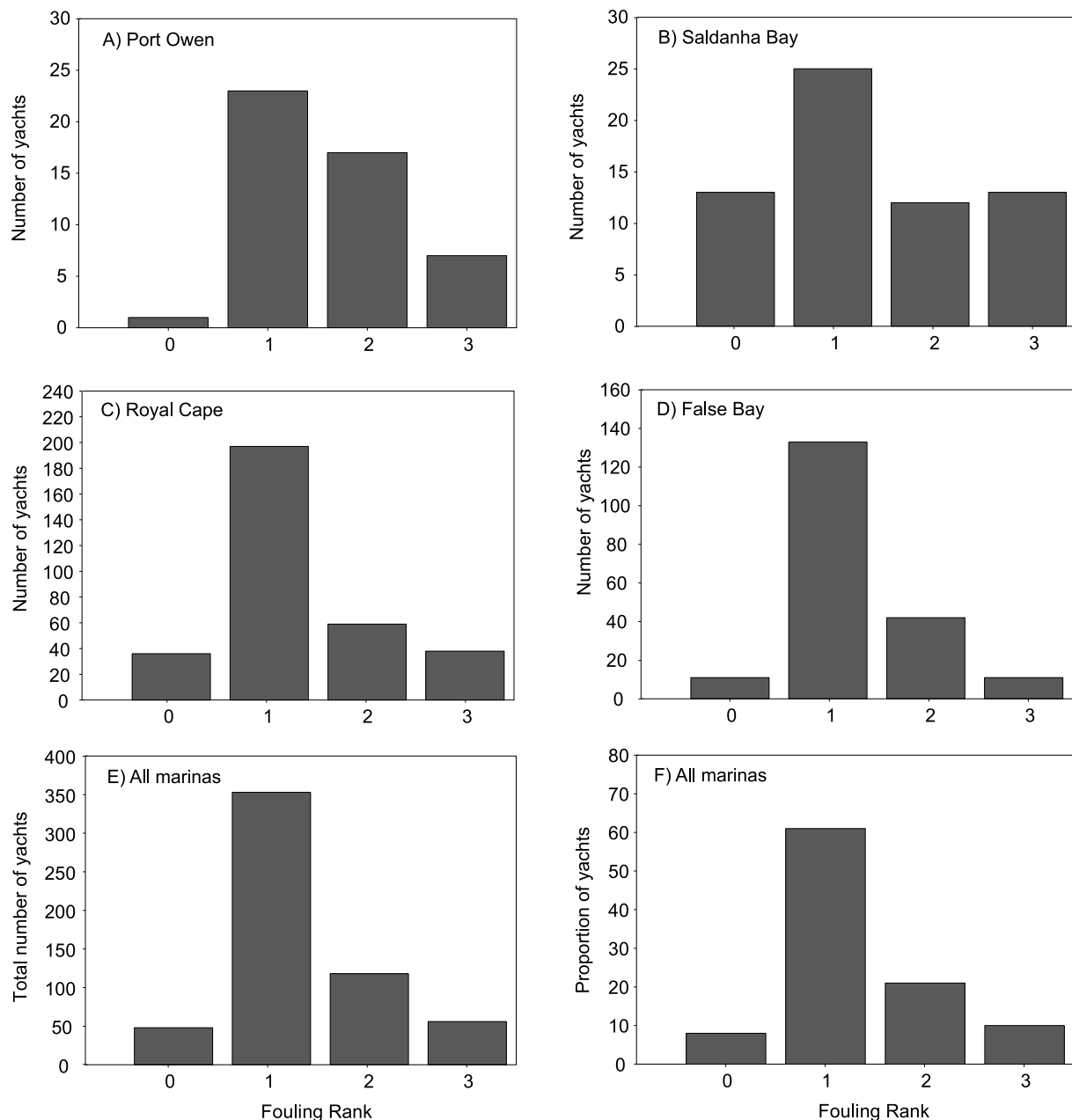


Fig. 1. The number of yachts observed in each Fouling Rank in A) Port Owen ($n = 48$), B) Saldanha Bay ($n = 63$), C) Royal Cape ($n = 330$) and D) False Bay Yacht Club ($n = 197$), along with E) the total number of yachts per Fouling Rank and F) the proportion of yachts per Fouling Rank for all marinas combined.

ROV) (for detailed results see supplementary electronic material Fig. S1). This relationship is reflected by an increase in species numbers with an increase in Fouling Rank, except for the niche areas where no relationship was observed. Nonetheless, there was notable variability in the number of alien species recorded in the various Fouling Ranks by all methods, especially when undertaken by divers. This is reflected in Spearman's correlation coefficients ranging from only 0.496 (Scrape Quadrat) to 0.674 (Photo Quadrat).

There was also a significant correlation between the mean percentage cover (Spearman's Rank Correlation, $r = 0.758$, $p < 0.001$; supplementary electronic material Fig. S2) and biomass (Spearman's Rank Correlation, $r = 0.747$, $p < 0.001$) of alien species and Fouling Rank. This was reflected in an increase in cover and biomass of alien species with an increase in Fouling Rank.

The best fit model for predicting the number of alien species was the saturated model and thus included all predictors. The number of alien

species recorded on yachts varied significantly among Fouling Ranks (Wald test, $X_3 = 100.25$, $p < 0.001$) and methods (Wald test, $X_5 = 134.11$, $p < 0.001$) (Fig. 2), with no significant interaction between these factors (Wald test, $X_{15} = 13.27$, $p = 0.58$). Although FR 0 and FR 1 did not differ significantly from one another and nor did FR 2 and FR 3 (Table 2), the general trend depicted an increase in alien species numbers with each successive increase in rank, with this pattern most clearly observed in the Meander, Transect and Quadrat methods (Fig. 3). The highest numbers of alien species (20 species) were recorded when divers collected scrape samples that were later sorted in the laboratory. The next most effective methods (i.e. all methods undertaken by divers) detected nine alien species. In contrast, the least effective methods for detecting alien species were the ROV transect and meander (a maximum of six taxa detected).

Suites of species detected depended on the method used (Psuedo-F = 2.913, $p < 0.01$) and the marina sampled (Psuedo-F = 53.279,

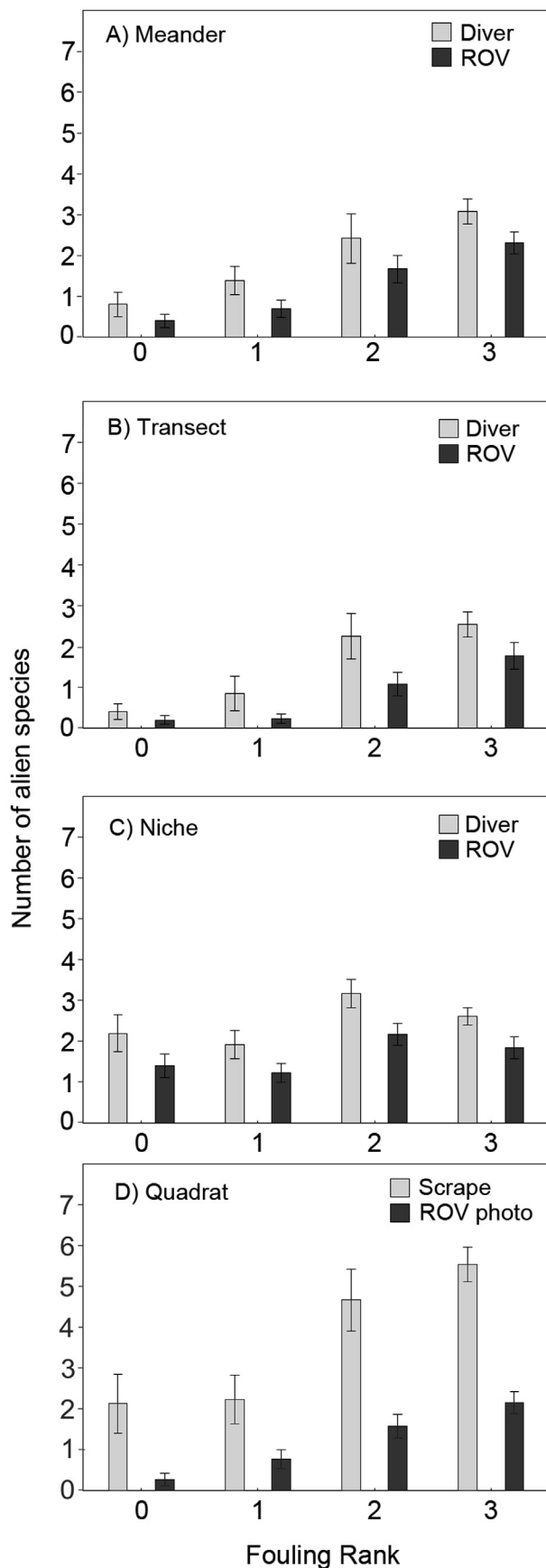


Fig. 2. Mean number of alien species (\pm SE) detected on yachts within each Fouling Rank for A) Meander, B) Transect, C) Niche and D) Quadrats methods as applied by diver or ROV.

$p < 0.01$). A significant interaction between these main effects (Pseudo- $F = 1.857$, $p < 0.01$) demonstrated that the effect of method differed among marinas, but this was driven solely by the lack of pairwise differences between methods at Royal Cape Yacht Club ($p > 0.05$). Notably, as a main effect and at all marinas except Royal Cape, the suite of species detected by scrape quadrats differed to that of all other methods ($p < 0.01$ in all cases). This is reflective of the fact that up to 55% of all species detected were unique to Scrape Quadrats regardless of Fouling Rank (supplementary electronic Table S3). At all Fouling Ranks, Scrape Quadrats allowed for the detection of most species and at low Fouling Ranks (0 and 1) the ROV Transect detected the least species.

3.3. Cost of sampling

The number of alien species detected (ANOVA, $F_7 = 7.5$, $p < 0.0001$) and the costs incurred (both in terms of time (ANOVA, $F_7 = 98.5$, $p < 0.0001$), and finances ANOVA, $F_7 = 27.8$, $p < 0.0001$)) differed significantly among sampling methods. The Scrape Quadrat was the most time-consuming method (Table 3, Fig. 3). It was also one of the most expensive methods, being one of five methods that cost more than \$60 per yacht (Fig. 3). However, this method detected significantly higher numbers of alien species than all of six other methods (Tukey HSD test, $p < 0.05$) except the Diver Niche method and was the most cost effective of all methods, when considering the cost: benefit ratio. The Diver Niche method was one of the least time-consuming methods, requiring less than 30 min per boat, and had the second best cost: benefit ratio. Overall the use of divers was least costly and time consuming. In contrast, all ROV methods detected fewest alien species, but incurred costs of more than \$60 per vessel with the ROV Transect method being least cost effective.

4. Discussion

Varying sampling methodologies have been used to detect alien species on recreational vessels (Floerl et al., 2005; Chapman et al., 2013; Zabin et al., 2014), without consideration of the effectiveness and costs of these methods. The present study showed that the collection of Scrape Quadrats was the most effective method at detecting alien species. Although this method was costly, it detected the highest numbers of alien species, with the lowest cost per species detected. Niche areas, such as the propeller, rudder, shaft, keel, vents and water intakes, were commonly fouled by alien species and thus the most effective approach for detecting alien species was a combination of scrape samples from the hull and the inspection of niche areas by a diver.

4.1. Fouling ranks

The majority of yachts sampled had at least a slime layer and $< 5\%$ fouling visible from the surface. In theory, these kinds of yachts represent a relatively low risk of transfer, since they are unlikely to carry macrofouling species (Floerl et al., 2005), an often used proxy for the number of alien species. However, whether a risk exists actually depends on the species under question and the densities at which they occur. Notably, this study found a significant positive correlation between species numbers and Fouling Rank for all methods considered, except for niche areas. This highlights that despite a clear relationship with fouling cover, Fouling Rank cannot accurately be used as a proxy for the number of alien species on yachts. This infers that yachts with higher levels of fouling should be targeted for monitoring purposes as the probability of detecting more alien species on such yachts is higher. However, the yachts that appear to have no fouling should not be overlooked, as they can support alien biota in their niche areas (Hopkins and Forrest, 2010). This lack of correlation is likely due to niche areas generally not being visible from the surface, which is the vantage point from which Fouling Rank is assigned.

Table 2

Main effect coefficient estimates and associated significance levels derived from the general mixed effect considering the effect of (a) Fouling Rank and (b) sampling method on the number of alien species recorded on yachts. ns not significant, * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

(a) Fouling rank	FR0	FR1	FR2	FR3
FR0	–			
FR1	0.606 ns	–		
FR2	1.133**	0.947**	–	
FR3	1.381***	1.075**	0.312 ns	–

b) Method	Diver Meander	ROV Meander	Diver Transect	ROV Transect	Diver Scrape Quadrat	ROV Photo Quadrat
Diver Meander	–					
ROV Meander	– 0.692 ns	–				
Diver Transect	– 0.694 ns	– 0.349 ns	–			
ROV Transect	– 1.383*	– 0.382 ns	– 1.300*	–		
Diver Scrape Quadrat	0.979**	1.078***	0.969**	1.460***	–	
ROV Photo Quadrat	– 1.100ns	0.049 ns	– 0.096 ns	0.379 ns	1.078***	–

Despite the dominance of lightly fouled yachts in this study, 11% of boats had high levels of fouling (i.e. FR 3). While yachts that have high levels of fouling and associated alien species may not pose a significant threat if they remain stationary in marinas (which is often the case with heavily fouled vessels that are typically uncared for), these yachts could serve as a source of high propagule pressure of alien species that might then be spread by yachts berthed nearby that travel frequently.

4.2. Sampling approaches and costs

This study clearly illustrates that the collection of scrape samples is the most effective method of detecting alien species. The outcome of applying various techniques to sample yachts for alien species indicated that, regardless of the method used, species numbers increased with Fouling Rank (except for niche areas), but variability in species numbers across the ranks was high. This is likely due to native species contributing to fouling cover but not alien diversity (Gittenberger and van der Stelt, 2011). At least in some cases, this variability weakens the premise that high levels of fouling are associated with high numbers of alien species.

Scrape samples detected 20 alien species overall, whereas all other methods were limited to large, conspicuous species, with the next most effective method detecting nine species. Thus, if the aim of a monitoring programme is to comprehensively detect alien species, then the use of Scrape Quadrats is the most appropriate method. This method was, however, one of the most expensive methods in terms of time and financial metrics but the most cost-effective when considering the cost per species detected. While it is important to be aware that the monetary cost calculations in this study may not be applicable in all regions, as the costs of some elements may vary, it is imperative to note that the relative costs among methods are unlikely to vary considerably. It is also noteworthy that the costs of the various methods should not be considered as additive if more than one method is to be implemented. This is because the cost of the ROV and the use of divers were calculated separately to enable the selection of a single method if needed. However, if a diver is hired for the day, the diver could undertake any or all of the methods requiring a diver and the cost of the diver will not be repeated. Similarly, if an ROV is purchased, then the ROV Meander, Transect, Niche and Photo Quadrat methods could all be undertaken, but the cost of the ROV would not be incurred for each method and only the time of the person operating the ROV would need to be accounted for. This suggests a reduced monetary cost in the field, but will concurrently increase the resources needed to process samples.

The Diver Niche method detected the most alien species after the collection of scrape samples. This method was not only effective at detecting alien species, but also had one of the lowest costs in terms of time and money, although the cost per species detected was slightly higher than scrape sampling. Niche areas of boats have previously been

highlighted as key areas for the attachment of alien species (Coutts and Taylor, 2004; Zabin et al., 2014). Species are thought to foul and to persist more readily in these areas as many are not anti-fouled (e.g. propellers and shafts) and are not as planar as the rest of the hull, thereby resulting in reduced exposure to hydrodynamic forces (Coutts and Taylor, 2004; Zabin et al., 2014). Due to the reduced costs associated with sampling niche areas in comparison to scrape samples, and the high number of alien species detected by this method, this approach offers good value for money to managers under circumstances of limited finances. Nonetheless, the combination of Scrape Quadrats and Diver Niche methods is considered the best approach to detect high numbers of alien species on recreational vessels. In addition to this, if smaller, inconspicuous species that could only be identified with the use of a microscope were to be targeted, then a scrape sample from niche areas could be used.

If the cost of collection of scrape samples is prohibitive then the next best method would be the Diver Meander. Although this method detected similar numbers to the Diver Transect, the Diver Meander method is recommended as light sensitive species (e.g. some ascidians) avoid the region close to the waterline where the transect is undertaken. Divers can also actively search for species across the entire hull area without investing much more time. In addition, as the diver will already be present in the water a combination of Diver Meander and Diver Niche would be an effective approach. The findings of the present study demonstrated that diver based methods were better at detecting alien species and were more affordable compared to the use of an ROV. Other constraints associated with the use of an ROV include the limited resolution that can be associated with such visual equipment, the fact that they require a shore-based power source or generator and the time required for set-up. Based on the experiences of this study, it is recommended that in future, divers should collect data on a slate, along with a diver-operated video attached to their head to record what they are seeing. In this way the cognitive ability of a diver, along with archivable high resolution footage that can be used by experts at a later stage (Lam et al., 2006) could be combined to obtain optimal benefit. Other studies have made use of such a diver operated video strategy (Boavida et al., 2016; Langlois et al., 2010; Pelletier et al., 2011). However, it should be noted that the use of visual surveys by divers detects fewer alien species compared to scrape samples and is dependent on visibility, a factor that varies temporally and among marinas. Thus, in regions where funding is limited, scientific divers should be used to detect and record alien species on yacht hulls. Such divers do, however, need to be trained to identify alien species. Should additional funding be available, the use of diver-operated video is encouraged, so that footage can later be reviewed if necessary. While the footage would increase the processing time, it would ground-truth the information that the divers record. Unfortunately, certain species that require dissection to confirm identification (e.g. several ascidian species) cannot

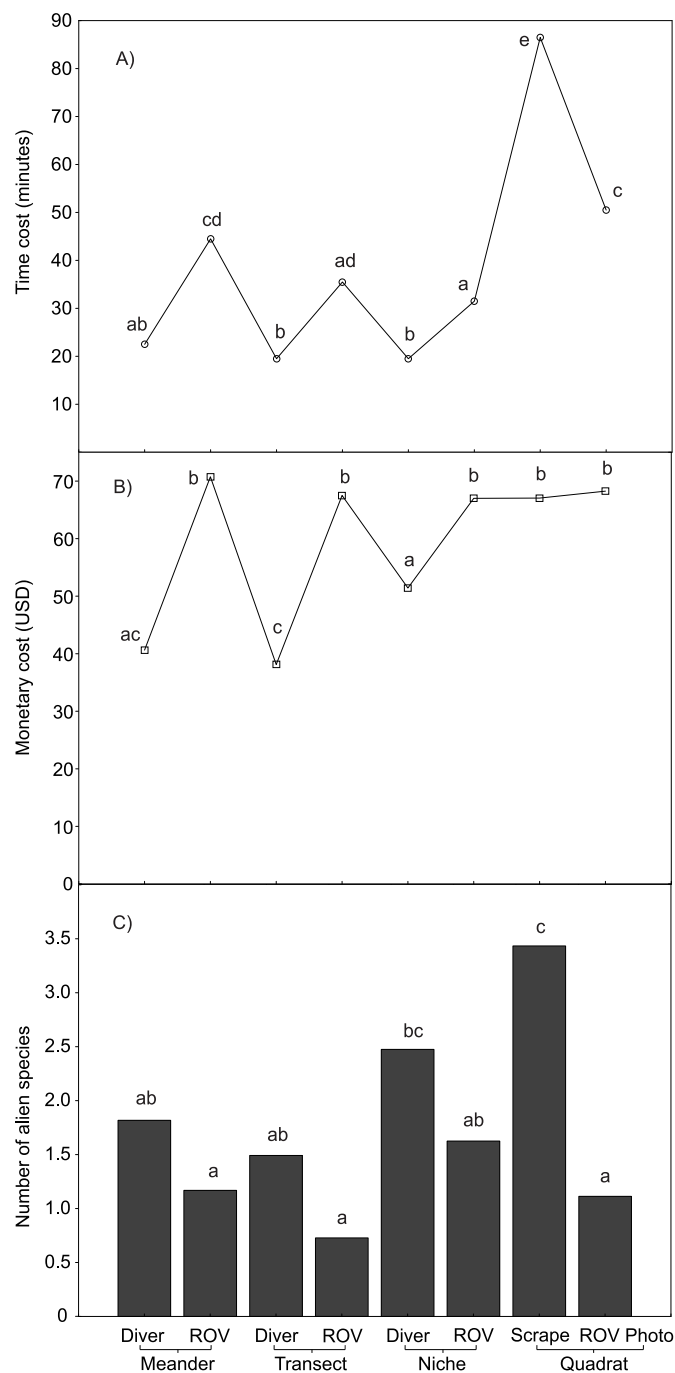


Fig. 3. The mean cost in terms of A) time and B) finances and the C) mean number of alien species recorded per boat. Data are combined for all four marinas. Refer to Table 3 for measures of variability. Shared lettering indicates no significant differences detected by posthoc Tukey HSD tests ($p < 0.05$).

be accounted for using this method. Thus the use of a diver would be the best option for rapid surveys, but the method that would be most likely to detect new species would be scrape samples. If resources do not allow for either of these methods, another approach could include the collection of targeted scrape samples with the use of a long pole and pocket net attached.

The usage of target lists as in this study has been applied in several other studies (Ashton et al., 2006; Minchin, 2007b; Minchin et al., 2016). Although target lists limit the potential species that can be detected, the use thereof requires a smaller search team (Marić et al., 2016) and facilitates training of divers and scientists. The use of target

Table 3

The mean (\pm SD) number of alien species, cost in terms of time (minutes) and money (USD) spent per boat and the cost: benefit ratio (cost per species detected), when applying the various methods. Underlined values depict the least desirable values in each category, while bold values are the most desirable.

Method	No. of species ($\bar{Y} \pm$ SD)	Minutes ($\bar{Y} \pm$ SD)	USD ($\bar{Y} \pm$ SD)	Cost: Benefit ratio
Diver Meander	1.8 ± 0.3	22.5 ± 5	40.8 ± 7.2	22.67
ROV Meander	1.2 ± 0.6	44.5 ± 4.6	<u>71.1 ± 0</u>	59.25
Diver Transect	1.5 ± 0.3	19.5 ± 5	38.3 ± 7.2	25.53
ROV Transect	<u>0.7 ± 0.7</u>	35.5 ± 4.6	67.8 ± 0	<u>96.86</u>
Diver Niche	2.5 ± 0.4	19.5 ± 5	51.7 ± 7.2	20.68
ROV Niche	1.6 ± 0.2	31.5 ± 4.6	67.3 ± 0	42.06
Scrape Quadrat	3.4 ± 1.2	<u>86.5 ± 1.3</u>	67.4 ± 7.2	19.82
ROV Quadrat	1.1 ± 0.7	50.5 ± 4.6	68.6 ± 0	62.36

species also ensures fast and cost-effective sampling compared to extensive surveys of all species present (Minchin et al., 2016). These lists can be made more inclusive by adding species that are on watchlists (i.e. species that have been identified as having the potential to arrive in the region but that have not yet been recorded) to account for the potential detection of new alien species (Hayes et al., 2002; Minchin, 2007b).

Based on the findings of the present study, we developed a framework to guide alien species monitoring of recreational vessels (Fig. 4). The intention of this framework is to enable managers to make informed decisions with regards to effectiveness and related costs of the various methods available for monitoring. The framework takes into consideration both the nature of the species that managers wish to detect and the associated costs. Species are classified based on their size, abundance and mobility. A method is recommended and the costs are reflected as Low, Moderate or High. “Low” costs involve only a diver using a target list to visually search for species. The diver would need to be a qualified scientific diver with the ability to identify all species on the target list. “Moderate” costs involve the use of a diver collecting data along with the use of a diver operated video and the collection of species from niche areas when an unusual species is noted or confirmation of a species identification is needed. “High” costs involve the collection of scrape samples from the hull and niche areas and the processing thereof. Although it should be noted that these costs are reduced to “Moderate” or even “Low” when fouling cover is low (i.e. FR 0 and FR 1). Nonetheless, scrape samples are the most cost efficient as they incur the least cost per species detected. When scrape sampling is not financially viable then other diver methods such as visual inspections of hull and niche areas are suggested. Additionally, costs may further be reduced if surveys are directed at specific, easily identifiable target groups. Notably the context of the monitoring programme also needs to be explicit when selecting sampling methods and considering their associated costs and benefits. For example, if the objective is to detect alien species in a particular location then it will be imperative to sample as many yachts as possible. In such an instance the best results are likely to be gained by divers collecting scrape samples from niche and hull areas but the costs will be high. In contrast if the objective is to prevent spread to other regions, only those yachts leaving the marina will need to be sampled prior to their departure and costs will be reduced. It is unlikely that long-term monitoring and once-off sampling programmes will require different approaches based on their duration. Rather sampling approaches should be selected to meet the desired management objectives, bearing in mind financial limitations.

5. Conclusions

The findings of this study highlight that in order to optimize the detection of marine alien species on the hulls of recreational vessels, diver-based sampling needs to be employed. The collection of scrape

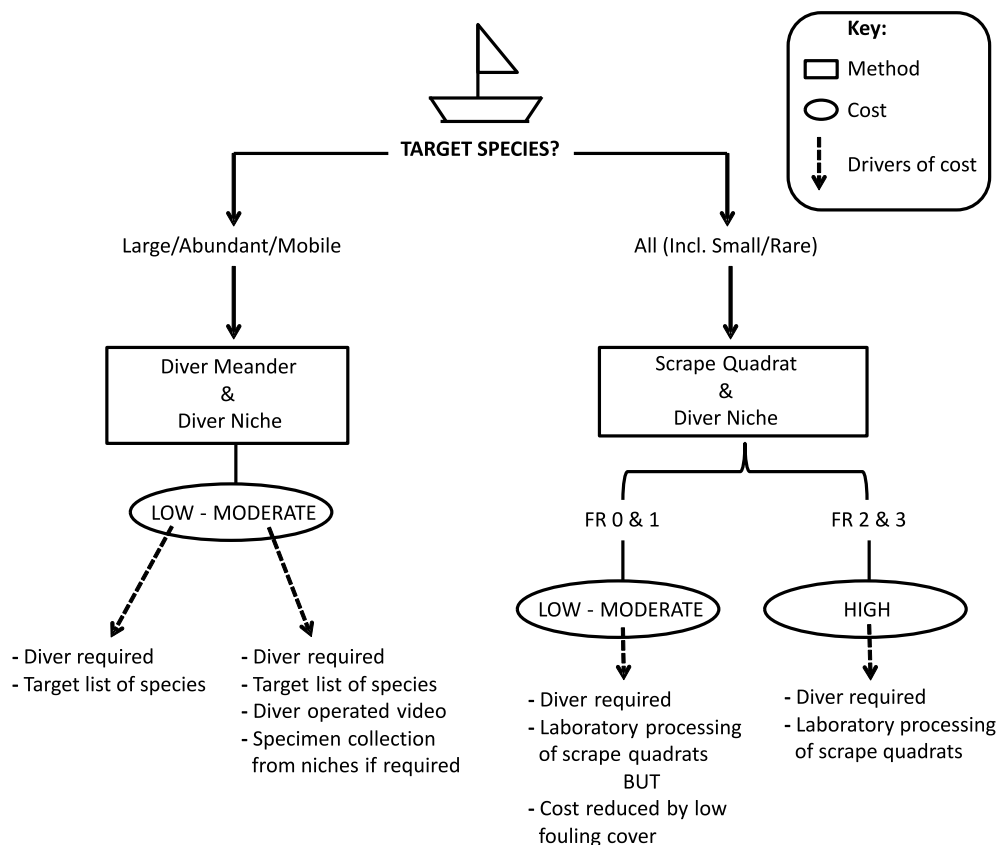


Fig. 4. Framework for selecting approaches for alien species monitoring on yacht fouling assemblages.

samples, while expensive, offers the most effective approach for alien species detection, followed by the observation of niche areas. However, depending on the financial constraints facing monitoring programmes, one of several diver methods can be applied, with the use of the framework provided, to detect alien species in fouling assemblages. The framework offers a rapid means of selecting appropriate sampling methodologies for alien species detection and can be applied to other recreational vessels. The findings of this study have therefore, advanced our understanding of sampling techniques for alien fouling assemblages on recreational vessels and provide a framework to support management authorities.

Declarations of interest

None.

Acknowledgments

This work was supported by the South African National Biodiversity Institute and the DST-NRF Centre of Excellence for Invasion Biology. The Research Dive Unit at the University of Cape Town is acknowledged for help in the field, especially Andrea Plos, Pieter Truter, Steven Horsley and Jessica Micklem. All fieldwork assistants are thanked, as are Katie Keanly and Sinethemba Kunene for help in the lab. We are grateful to three anonymous reviewers for their comments on an earlier version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2018.09.063>.

References

- Adebayo, A.A., Zhan, A., Bailey, S.A., MacIsaac, H.J., 2013. Domestic ships as a potential pathway of nonindigenous species from the Saint Lawrence river to the great Lakes. *Biol. Invasions* 16, 793–801. <https://doi.org/10.1007/s10530-013-0537-5>.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070>.
- Ashton, G., Boos, K., Shucksmith, R., Cook, E., 2006. Risk assessment of hull fouling as a vector for marine non-natives in Scotland. *Aquat. Invasions* 1, 214–218. <https://doi.org/10.3391/ai.2006.1.4.4>.
- Bax, N., Hayes, K., Marshall, A., Parry, D., Thresher, R., 2002. Man-made marinas as sheltered islands for alien marine organisms: establishment and eradication of an alien invasive marine species. In: Veitch, C.R., Clout, M.N. (Eds.), *Turning the Tide: the Eradication of Invasive Species*. IUCN SSC Invasive Species Specialist Group, IUCN, Gland, Switzerland and Cambridge, UK, pp. 26–39.
- Beaumont, J.C., Brown, C.J., Sayer, M.D.J., 2007. Evaluation of techniques used in the assessment of subtidal epibiotic assemblage structure. *Biofouling* 23 (5), 343–356. <https://doi.org/10.1080/08927010701492219>.
- Boavida, J., Assis, J., Reed, J., Serrão, E.A., Gonçalves, J.M.S., 2016. Comparison of small remotely operated vehicles and diver-operated video of circalittoral benthos. *Hydrobiologia* 766, 247–260. <https://doi.org/10.1007/s10750-015-2459-y>.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24 (3), 127–135. <https://doi.org/10.1016/j.tree.2008.10.008>.
- Brine, O., Hunt, L., Costello, M.J., 2013. Marine biofouling on recreational boats on swing moorings and berths. *Manag. Biol. Invasion.* 4, 327–341. <https://doi.org/10.3391/mbi.2013.4.4.07>.
- Chapman, J.W., Breitenstein, R.A., Carlton, J.T., 2013. Port-by-port accumulations and dispersal of hull fouling invertebrates between the Mediterranean sea, the Atlantic ocean and the Pacific ocean. *Aquat. Invasions* 8 (3), 249–260. <https://doi.org/10.3391/ai.2013.8.3.01>.
- Chan, F.T., Briski, E., 2017. An overview of recent research in marine biological invasions. *Mar. Biol.* 164, 121. <https://doi.org/10.1007/s00227-017-3155-4>.
- Clarke Murray, C., 2012. The Role of Recreational Boating in the Introduction and Spread of Marine Invasive Species. PhD Dissertation. The University of British Columbia, Vancouver, British Columbia.
- Clarke Murray, C., Pakhomov, E.A., Therriault, T.W., 2011. Recreational boating: a large unregulated vector transporting marine invasive species. *Divers. Distrib.* 17, 1161–1172. <https://doi.org/10.1111/j.1472-4642.2011.00798.x>.
- Coutts, A.D.M., Dodgson, T.J., 2007. The nature and extent of organisms in vessel sea-chests: a protected mechanism for marine bioinvasions. *Mar. Pollut. Bull.* 54,

- 875–886. <https://doi.org/10.1016/j.marpolbul.2007.03.011>.
- Coutts, A.D.M., Taylor, M.D., 2004. A preliminary investigation of biosecurity risks associated with biofouling on merchant vessels in New Zealand. *New Zeal. J. Mar. Fresh.* 38, 215–229. <https://doi.org/10.1080/00288330.2004.9517232>.
- Davidson, I.C., Brown, C.W., Sytsma, M.D., Ruiz, G.M., 2009. The role of containerships as transfer mechanisms of marine biofouling species. *Biofouling* 25, 645–655. <https://doi.org/10.1080/08927010903046268>.
- Davidson, I.C., Zabin, C.J., Chang, A.L., Brown, C.W., Sytsma, M.D., Ruiz, G.M., 2010. Recreational boats as potential vectors of marine organisms at an invasion hotspot. *Aquat. Biol.* 11, 179–191. <https://doi.org/10.3354/ab00302>.
- Floerl, O., Inglis, G.J., 2005. Starting the invasion pathway: the interaction between source populations and human transport vectors. *Biol. Invasions* 7, 589–606. <https://doi.org/10.1007/s10530-004-0952-8>.
- Floerl, O., Inglis, G.J., Hayden, B.J., 2005. A risk-based predictive tool to prevent accidental introductions of nonindigenous marine species. *Environ. Manag.* 35, 765–778. <https://doi.org/10.1007/s00267-004-0193-8>.
- Galil, B.S., Boero, F., Campbell, M.L., Carlton, J.T., Cook, E., Fraschetti, S., Gollasch, S., Hewitt, C.L., Jelmer, A., Macpherson, E., Marchini, A., McKenzie, C., Minchin, D., Occhipinti-Ambrogi, A., Ojaveer, H., Olenin, S., Piraino, S., Ruiz, G.M., 2015. 'Double trouble': the expansion of the Suez canal and marine bioinvasions in the Mediterranean sea. *Biol. Invasions* 17, 973–976. <https://doi.org/10.1007/s10530-014-0778-y>.
- Gittenberger, A., van der Stelt, R.C., 2011. Artificial structures in harbours and their associated ascidian fauna. *Aquat. Invasions* 6 (4), 413–420. <https://doi.org/10.3391/ai.2011.6.4.06>.
- Grosholz, E.D., Crafton, R.E., Fontana, R.E., Pasari, J.R., Williams, S.L., Zabin, C.J., 2015. Aquaculture as a vector for marine invasions in California. *Biol. Invasions* 17, 1471–1484. <https://doi.org/10.1007/s10530-014-0808-9>.
- Hayes, K.R., McEnnulty, F.R., Sliwa, C., 2002. Identifying Potential Marine Pests: an Inductive Approach. Centre for Research on Introduced Marine Pests Marine Research, Hobart.
- Hewitt, C.L., Campbell, M.L., Schaffelke, B., 2007. Introductions of seaweeds – accidental transfer pathways and mechanisms. *Bot. Mar.* 50, 326–337. <https://doi.org/10.1515/BOT.2007.038>.
- Hewitt, C.L., Gollasch, S., Minchin, D., 2009. The vessel as a vector - biofouling ballast water and sediments. In: Rilov, G., Crooks, J.A. (Eds.), *Biological Invasions in Marine Ecosystems*. Springer-Verlag, Berlin, pp. 117–131.
- Holmberg, R.J., Tlustý, M.F., Futoma, E., Kaufman, L., Morris, J.A., Rhyne, A.L., 2015. The 800-pound grouper in the room: Asymptotic body size and invasiveness of marine aquarium fishes. *Mar. Pol.* 53, 7–12. <https://doi.org/10.1016/j.marpol.2014.10.024>.
- Hopkins, G.A., Forrest, B.M., 2010. Challenges associated with pre-border management of biofouling on oil rigs. *Mar. Pollut. Bull.* 60, 1924–1929. <https://doi.org/10.1016/j.marpolbul.2010.07.015>.
- Lam, K., Shin, P.K.S., Bradbeer, R., Randall, D., Ku, K.K.K., Hodgson, P., Cheung, S.G., 2006. A comparison of video and point intercept transect methods for monitoring subtropical coral communities. *J. Exp. Mar. Biol. Ecol.* 333, 115–128. <https://doi.org/10.1016/j.jembe.2005.12.009>.
- Langlois, T.J., Harvey, E.S., Fitzpatrick, B., Meeuwig, J.J., Shedrawi, G., Watson, D.L., 2010. Cost-efficient sampling of fish assemblages: comparison of baited video stations and diver video transects. *Aquat. Biol.* 9, 155–168. <https://doi.org/10.3354/ab00235>.
- Lee, J.E., Chown, S.L., 2009. Temporal development of hull-fouling assemblages associated with an Antarctic supply vessel. *Mar. Ecol. Prog. Ser.* 386, 97–105. <https://doi.org/10.3354/meps08074>.
- Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., Bazzaz, E.A., 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol. Appl.* 10, 689–710. [https://doi.org/10.1890/1051-0761\(2000\)010\[0689:BICEGC\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2).
- Mallet, D., Pelletier, D., 2014. Underwater video techniques for observing coastal marine biodiversity: a review of sixty years of publications (1952–2012). *Fish. Res.* 154, 44–62. <https://doi.org/10.1016/j.fishres.2014.01.019>.
- Marchi, M., Jørgensen, S.E., Bécarea, E., Corsi, I., Marchettini, N., Bastianoni, S., 2011. Dynamic model of Lake Chozas (Léon, NW Spain) – decrease in eco-exergy from clear to turbid phase due to introduction of exotic crayfish. *Ecol. Model.* 222, 3002–3010. <https://doi.org/10.1016/j.ecolmodel.2011.04.016>.
- Marić, M., Ferrario, J., Marchini, A., Occhipinti-Ambrogi, A., Minchin, D., 2016. Rapid assessment of marine non-indigenous species on mooring lines of leisure craft: new records in Croatia (eastern Adriatic Sea). *Mar. Biodivers.* 47 (3), 949–956. <https://doi.org/10.1007/s12526-016-0541-y>.
- Minchin, D., 2007a. Aquaculture and transport in a changing environment: overlap and links in the spread of alien biota. *Mar. Pollut. Bull.* 55, 302–313. <https://doi.org/10.1016/j.marpolbul.2006.11.017>.
- Minchin, D., 2007b. Rapid coastal survey for targeted alien species associated with floating pontoons in Ireland. *Aquat. Invasions* 2 (1), 63–70. <https://doi.org/10.3391/ai.2007.2.1.8>.
- Minchin, D., Floerl, O., Savini, D., Occhipinti-Ambrogi, A., 2006. Small craft and the spread of exotic species. In: Davenport, J., Davenport, J. (Eds.), *The Ecology of Transportation: Managing Mobility for the Environment*. Springer, Dordrecht, pp. 99–118.
- Minchin, D., Olenin, S., Liu, T.-K., Cheng, M., Huang, S.-C., 2016. Rapid assessment of target species: byssate bivalves in a large tropical port. *Mar. Pollut. Bull.* 112, 177–182. <https://doi.org/10.1016/j.marpolbul.2016.08.023>.
- Needles, L.A., Wendt, D.E., 2013. Big changes to a small bay: introduced species and long-term compositional shifts to the fouling community of Morro Bay (CA). *Biol. Invasions* 15, 1231–1251. <https://doi.org/10.1007/s10530-012-0362-2>.
- Pelletier, D., Leleu, K., Mou-Tham, G., Guillemot, N., Chabanet, P., 2011. Comparison of visual census and high definition video transects for monitoring coral reef fish assemblages. *Fish. Res.* 107, 84–93. <https://doi.org/10.1016/j.fishres.2010.10.011>.
- Peters, K., Griffiths, C.L., Robinson, T.B., 2014. Patterns and drivers of marine bioinvasions in eight Western Cape harbours, South Africa. *Afr. J. Mar. Sci.* 36, 49–57. <https://doi.org/10.2989/1814232X.2014.890669>.
- Peters, K., Sink, K., Robinson, T.B., 2017. Raising the flag on marine alien fouling species. *Manag. Biol. Invasions* 8, 1–11. <https://doi.org/10.3391/mbi.2017.8.1.01>.
- Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., Vilà, M., 2013. Impacts of biological invasions: what's what and the way forward. *Trends Ecol. Evol.* 28, 58–66. <https://doi.org/10.1016/j.tree.2012.07.013>.
- Wasson, K., Zabin, C.J., Bedinger, L., Diaz, M.C., Pearse, J.S., 2001. Biological invasions of estuaries without international shipping: the importance of intraregional transport. *Biol. Conserv.* 102, 143–153. [https://doi.org/10.1016/S0006-3207\(01\)00098-2](https://doi.org/10.1016/S0006-3207(01)00098-2).
- Willan, R.C., Russel, B.C., Murfet, N.B., Moore, K.L., McEnnulty, F.R., Horner, S.K., Hewitt, C.L., Dally, G.M., Campbell, M.L., Bourke, S.T., 2000. Outbreak of *Mytilopsis sallei* (Récluz, 1849) (Bivalvia: Dreissenidae) in Australia. *Molluscan Res.* 20 (2), 25–30. <https://doi.org/10.1080/13235818.2000.106737>.
- Williams, S.L., Davidson, I.C., Pasari, J.R., Ashton, G.V., Carlton, J.T., Crafton, R.E., Fontana, R.E., Grosholz, E.D., Miller, A.W., Ruiz, G.M., Zabin, C.J., 2013. Managing multiple vectors for marine invasions in an increasingly connected world. *Bioscience* 63, 952–966. <https://doi.org/10.1525/bio.2013.63.12.8>.
- Zabin, C.J., Ashton, G.V., Brown, C.W., Davidson, I.C., Sytsma, M.D., Ruiz, G.M., 2014. Small boats provide connectivity for nonindigenous marine species between a highly invaded international port and nearby coastal harbors. *Manag. Biol. Invasions* 5, 97–112. <https://doi.org/10.3391/mbi.2014.5.2.03>.